ICI AMERICAS INC.
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Study Title

The Determination of Residues of Paraquat (PP148) in Animal Products; A High Performance Liquid Chromatographic Method

Data Requirement

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Performing Laboratory

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Bracknell, Berkshire, UK

Laboratory Project ID

ICI Residue Analytical Method 4B

STATEMENT OF DATA CONFIDENTIALITY CLAIMS

[X] No claim of confidentiality under FIFRA Section 10(d)(1)(A), (B), or (C)

STATEMENT OF NO DATA CONFIDENTIALITY CLAIMS

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA Section 10(d)(1)(A), (B), or (C).

Company: ICI AMERICAS INC.

Company Agent: B. J. Kaminski Date: December 20, 1988

Senior Pesticide Regulatory Specialist
Title

B J Kaminski
Signature

GOOD LABORATORY PRACTICE STATEMENT

[X] This document is not subject to the requirements of 40 CFR Part 160.

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ICI AGROCHEMICALS RESIDUE ANALYTICAL METHOD 4B.

THE DETERMINATION OF RESIDUES OF PARAQUAT(PP148) IN ANIMAL PRODUCTS

A High Performance Liquid Chromatographic Method

Authors : M Earl, A D Boseley

Study Director : M Earl

Date of Issue : November 1988

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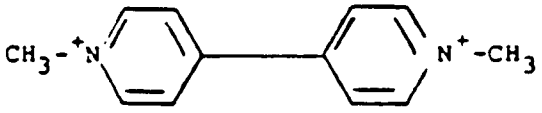
Common Name : Paraquat

Chemical Name (IUPAC) : 1,1'-dimethyl-4,4'-bipyridinium ion



Molecular Formula : $C_{12}H_{14}N_2$

Code Number : PP148

Molecular Weight : 186

This method cancels and replaces PPRAM 4 dated 30 April 1986.

1. SCOPE

The analytical procedures described are suitable for the determination of residues of the herbicide paraquat in eggs and animal tissues.

2. SUMMARY

Samples are extracted by homogenisation with 10% trichloroacetic acid solution.

The centrifuged homogenate is percolated through a column of cation-exchange resin which retains the paraquat and some of the natural tissue constituents. The column is washed with water, 2.5% ammonium chloride solution and water to remove endogenous materials and the paraquat is then eluted with saturated ammonium chloride solution.

Final quantitative determination is by high performance liquid chromatography using U.V. detection.

3. PROCEDURE

3.1 Sample Preparation

Tissue samples should be removed from the deep freeze and allowed to stand at room temperature for approximately 30 minutes until it is possible for them to be sliced prior to mincing. The mincing/chopping should be continued until a truly homogenous sample is obtained.

Samples which are removed from the deep freezer having previously been homogenised, should be allowed to thaw for the minimum period only before breaking up and weighing out; this ensures that no partition of the endogenous water content can occur prior to analysis.

Egg samples should be thoroughly thawed and mixed before sub-sampling.

3.2 **Extraction and Chromatographic Separation**

(a) Thoroughly mix the sample and weigh a representative aliquot (25 g) into a centrifuge bottle. Add trichloroacetic acid solution (50 ml, 10%) and homogenise for 5 minutes.

(b) Centrifuge the homogenate at 3000 rpm for 10 minutes and transfer the supernatant to a 250 ml round bottom flask. Re-extract by homogenising the tissue sample with two further portions of trichloroacetic acid solution (50 ml) and after each centrifugation combine the supernatants in the 250 ml round bottom flask.

NB. If the samples have a high fat content the TCA extract can be partitioned with hexane (100 ml). Discard the hexane before percolating the TCA extract through the ion-exchange resin.

(c) While the samples are being centrifuged the ion-exchange columns are prepared as follows: Wash 3.5 g of resin with water into a burette (25 ml) containing a glass wool plug placed near the stopcock. Pass successively through the column at the rate of 5 ml/min saturated sodium chloride solution (20 ml) and water (50 ml). Prepare a separate column for each sample.

(d) Filter the supernatants from centrifugation through a glass fibre filter paper to remove fine particulates from extraction.

(e) Dilute the combined trichloroacetic acid extracts to 500 ml with deionised water and allow the solution to percolate through a prepared resin column from 3.2 (c) above at a flow rate of 5-10 ml/min.

(f) Remove the funnel and wash the column at a flow rate of 3-4 ml/min successively with water (25 ml), 2.5% (w/v) ammonium chloride solution (100 ml) and water (50 ml). (The process can be left overnight provided the resin column has been covered with water).

(g) Elute the paraquat from the column with saturated ammonium chloride solution at a flow rate of about 1 ml/min. Collect the first 50 ml of the eluent in a 50 ml volumetric flask and mix.

NB. The recovery of the paraquat from the resin column will be adversely affected if the flow rate of the eluent exceeds 1.0 ml/min.

4. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC).

These analytical conditions will depend upon the equipment and columns available. The following conditions have been found satisfactory in this laboratory.

4.1 Reverse Phase Ion Pair Chromatography

Analytical conditions for liquid chromatograhy:-

Equipment	: Waters 501 pump Waters Intelligent Sample Processor model 712 Waters M-481 LC-UV detector
Column	: Spherisorb S5P 25.0 cm x 4.6 mm i.d.
Mobile Phase	: Water:Methanol 90:10 + 0.01 mol dm^{-3} sodium-1-octanesulphonate + 0.8% orthophosphoric acid + 1% diethylamine
Flow rate	: 1.5 ml min^{-1}
Detection	: 258 nm
Chart speed	: 300 mm hr^{-1}
Retention time	: 9 mins

4.2 Calculation of Paraquat Residue Results

a) Make repeated injections (350 µl), of an analytical standard solution of paraquat into an HPLC operated under the conditions described above. When a consistent response is obtained measure the peak height (or area) for the standard.

b) Inject 350 µl of the sample solution and similarly measure the response at the retention time of paraquat.

c) Reinject the standard solution after a maximum of six injections of sample solutions.

d) Calculate the residue in the sample by a simple proportion calculation i.e.

$$\text{Residue (mg kg}^{-1}\text{)} = \frac{\text{response sample}}{\text{response standard}} \times \frac{\text{conc. standard}}{\text{conc. sample}} \times \frac{\text{injection volume of standard}}{\text{injection volume of sample}}$$

e) Correct the measured residue value for the mean percentage recovery of fortified control samples i.e. for a mean 80% recovery, corrected residue = measured residue x $\frac{100}{80}$

Note : in the case where laboratory data systems/computing integrators are used the computer algorithm may adopt a slightly different method for calculation of results. For example, the VG-LS Multichrom laboratory data system uses the relative detector response factor calculated from an analytical standard solution as the basis for calculation of results. The final calculated result is, of course, the same as the above manual calculation.

5. CONTROL AND RECOVERY EXPERIMENTS

At least one untreated sample must be analysed alongside any set of samples, using exactly the same method. This ensures that no contamination of the samples occurred prior to, or during, the analysis.

Recovery experiments should be carried out by adding known amounts of paraquat to untreated samples prior to the acid digestion stage. The amount added should be similar to the amounts that are expected in the treated samples.

In these laboratories using this procedure recoveries of between 75% and 90% of the added paraquat are expected.

6. LIMIT OF DETERMINATION

The true limit of determination of paraquat residues will give chromatographic response of at least 4x the background noise at the retention time of paraquat and precision of reproducibility of better than $\pm$ 5%. In these laboratories the limit of determination has been set at 0.005 mg kg^{-1} for egg and animal tissue samples.

7. METHOD VALIDATION STUDIES

7.1 Controls

In these laboratories to date the method has been applied to the analysis of eggs, muscle, skin, kidney, liver and fat from bovine, ovine and poultry species.

No endogenous materials from these substrateshave been observed to interfere with paraquat during the final chromatographic determination step.

7.2 Storage Stability Studies

Samples of eggs and tissues for residue analysis are stored deep frozen prior to analysis. Whilst this period is kept to a minimum it is necessary to demonstrate the effect of storage at <-18°C upon paraquat residues. Residues of paraquat in eggs and muscle tissue have been shown in two studies, to be stable for at least six and five months respectively (References 1 and 2).

8. REFERENCES

1. Earl, M., Boseley A. D. - Paraquat:Storage Stability of Residues in Frozen Eggs. ICI AgrochemicalsReport M4847B.

2. Earl. M., Boseley A. D. - Paraquat:Storage Stability of the Residue in Frozen Hen Muscle Tissue. ICI Agrochemicals Report M4846B.

APPENDIX

1. Apparatus

(a) Equipment which can be used for the initial preparation of samples ie, Hobart laboratory mincer.

(b) Silverson homogeniser. Available from Silverson Machines Ltd, Chesham, Bucks.

(c) Centrifuge with capacity for 250 ml centrifuge bottles.

(d) Glass columns for chromatography of 1.0 cm i.d. and 50 cm long (25 ml burettes are suitable).

(e) High Performance Liquid Chromatograph . e.g. Waters Model 501 pump WISP 712 autosampler and Waters M-481 LC-UV detector or equivalent instruments.

(f) HPLC column S5P 25.0 cm x 4.6 mm i.d available from Hichrom Ltd, Reading, Berkshire, UK.

2. Reagents

(a) Trichloroacetic acid : Lancaster Synthesis Ltd, Morecambe, UK.

(b) Granular sodium chloride : May and Baker Ltd., Dagenham, UK.

(c) Cation-exchange resin : Particle size 0.15 - 0.30 mm. 52 - 100 mesh, sodium form. BDH Chemicals Ltd., Poole, UK.

(d) Ammonium chloride : May and Baker Ltd, Dagenham, UK.

(e) Solvents : redistilled hexane and methanol. Rathburn Chemicals Ltd, Walkerburn, Scotland.

(f) Diethylamine : Lancaster Synthesis Ltd., Morecambe, UK.

(g) Orthophosphoric acid : BDH Chemicals Ltd., Poole, UK.

(h) Sodium-1-octanesulphonate : HPLC grade. Lancaster Synthesis Ltd., Morecambe, UK.

(i) A sample of paraquat dichloride of known purity.

3. Hazards

The following information is included as an indication to the analyst of the nature and hazards of the reagents used in this procedure. if in doubt, consult the appropriate safety manual (eg ICI Laboratory Safety Manual) containing recommendations and procedures for handling chemicals, and a monograph such as 'Hazards in the Chemical Laboratory', edited by G D Muir, The Chemical Society, London.

TRICHLOROACETIC ACID

Serious risk of poisoning by inhalation, swallowing or skin contact.
Causes severe burns.

PARAQUAT

Toxic by ingestion
Harmful dust.
Avoid contact with eyes, skin and mouth. Avoid breathing dust.
Wash hands and exposed skin before meals and after work.

Ingestion of paraquat should be regarded as a dire emergency and action taken immediately. Details of remedial action/antidotes should be available in the laboratory.

HEXANE

Extremely flammable
Avoid breathing vapour
(TLV 100 ppm or 360 mgm^{-3})

METHANOL

Highly flammable
Toxic by inhalation and if swallowed
Avoid breathing vapour
Avoid contact with skin and eyes
(TLV 260 mgm^{-3})

DIETHYLAMINE

Harmful vapour
Harmful by skin absorption
Harmful if taken internally
Highly flammable
Avoid breathing vapour or contact with skin and eyes
(TLV 10 ppm or 30 mgm^{-3})

ORTHOPHOSPHORIC ACID

Causes burns
Avoid contact with eyes and skin
(TLV 1 mg m^{-3})

4. PREPARATION OF ANALYTICAL STANDARDS

Weigh out accurately, using a five figure balance, sufficient paraquat dichloride to allow dilution to give a 250 $\mu g cm^{-3}$ paraquat stock solution in a volumetric flask. Make serial dilutions of this stock solution to give 10 $\mu g\ cm^{-3}$, 1 and 0.1 $\mu g\ cm^{-3}$ paraquat standard solutions in saturated ammonium chloride solution. These standards should be used for the fortification of recovery samples and as standards for HPLC analysis.

These solutions are stable under normal laboratory conditions provided that they are not exposed to sunlight for long periods.

It is recommended that the following handling precautions should be taken when weighing the analytical standard materials.

1. Ensure good ventilation
2. Wear gloves and laboratory coat
3. Prevent inhalation and contact with mouth.
4. Wash any contaminated area immediately.

APPENDIX 5

Typical High Performance Liquid Chromatograms for Paraquat Residue Determination in Animal Products

5.1 **Paraquat Determination in Eggs and Muscle (Hen)**

Figure 1 : 0.1 μg cm^{-3} Paraquat

Figure 2 : Untreated egg sample at 0.5 g cm^{-3}

Figure 3 : Untreated egg sample + 0.1 $mgkg^{-1}$ at 0.5 g cm^{-3}

Figure 4 : Treated egg sample at 0.5 g cm^{-3}. Residue 0.04 mg kg^{-1}

Figure 5 : Untreated muscle sample at 0.5 g cm^{-3}

Figure 6 : Untreated muscle sample + 0.1 mg kg^{-1} at 0.5 g cm^{-3}

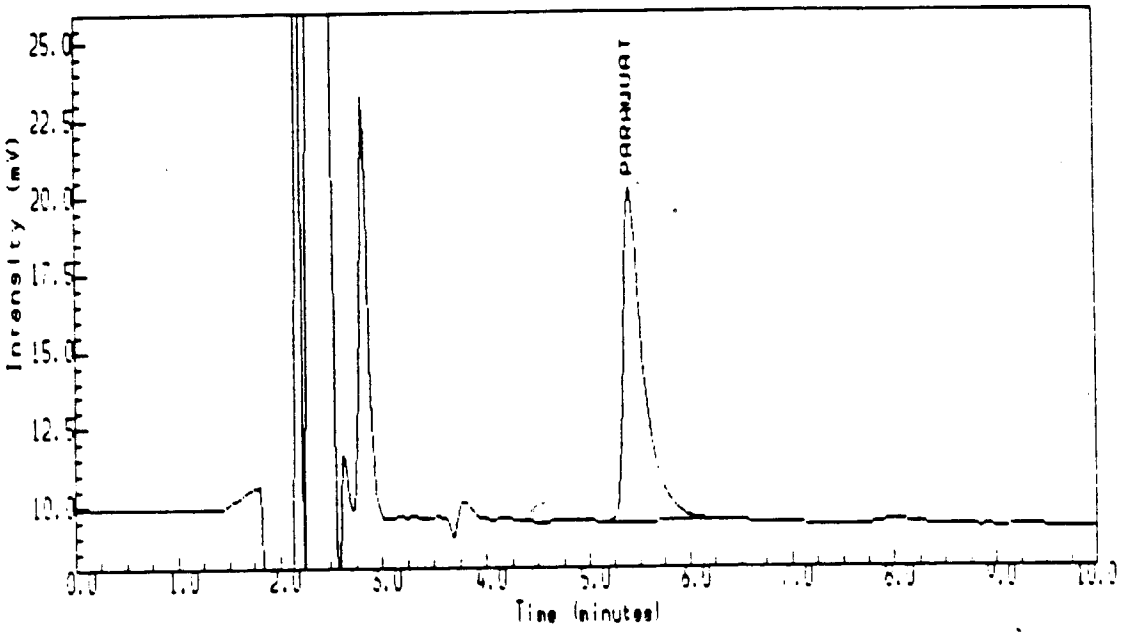
Figure 7 : Treated muscle sample at 0.5 g cm^{-3}. Residue 0.02 mg kg^{-1}

Figure 1

[RESIDUE] 23 IL277B,1,1
Reported on 14-NOV-1988 at 11:16

Injection Report

Acquired on 4-Jun-1988 at 11:26



Sample Name : STD
Sample Id : 0
Sample Type : Standard Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	5.419	147587	0.100	[illegible]
Residual		0	N/A	
Total		147587	0.100	

MISSING PEAKS

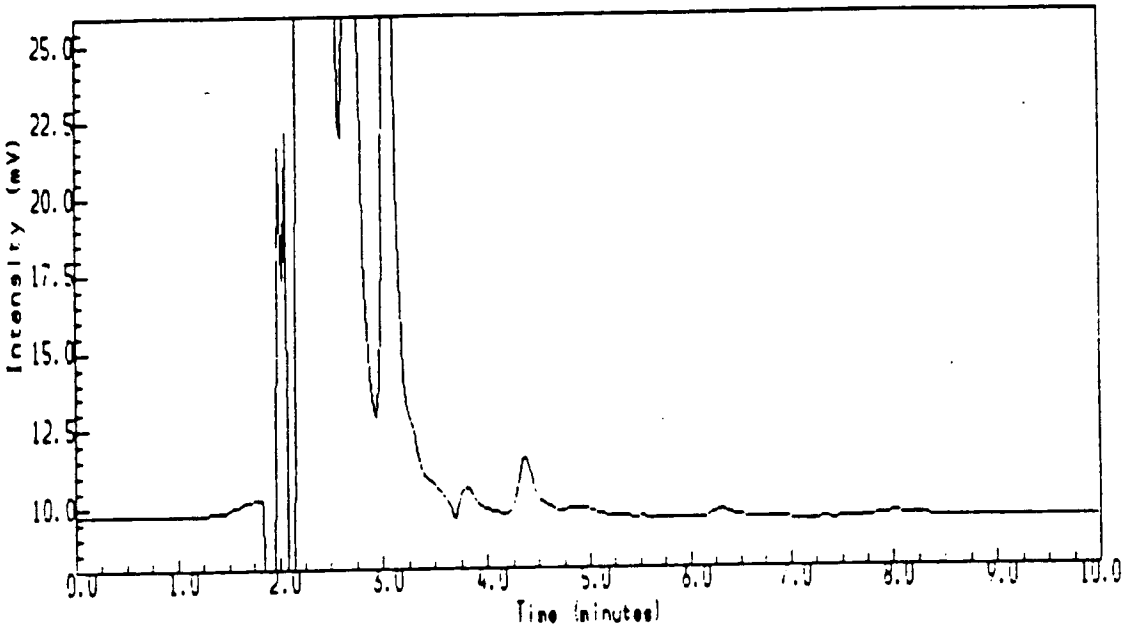
No missing peaks.

Figure 2

[RESIDUE] 23 IL277B,2,1
Reported on 14-NOV-1988 at 11:18

Injection Report

Acquired on 4-Jun-1988 at 11:57



Sample Name : C2639/88
Sample Id : 0
Sample Type : Sample Amount=1.00000
Bottle No : 1

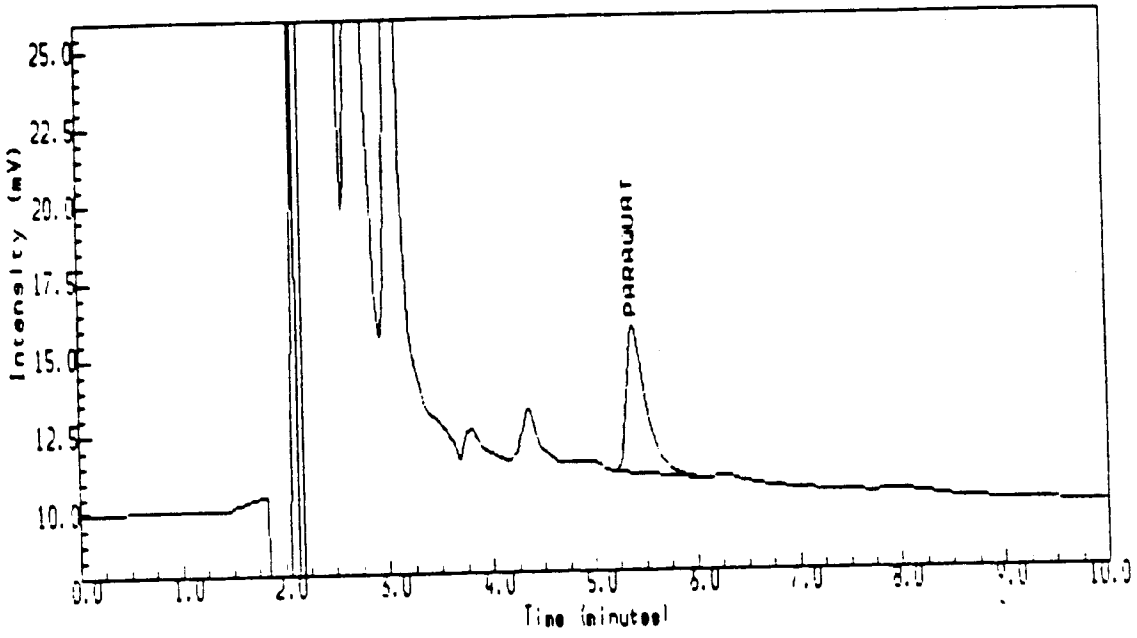
No peaks detected

FIgure 3

[RESIDUE] 23 IL277B,3,1
Reported on 14-NOV-1988 at 11:19

Injection Report

Acquired on 4-Jun-1988 at 12:27



Sample Name : R1 2639/88 +0.1
Sample Id : 0
Sample Type : Sample Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	5.397	61156	0.083	PARAQUAT
Residual		0	N/A	
Total		61156	0.083	

MISSING PEAKS

No missing peaks.

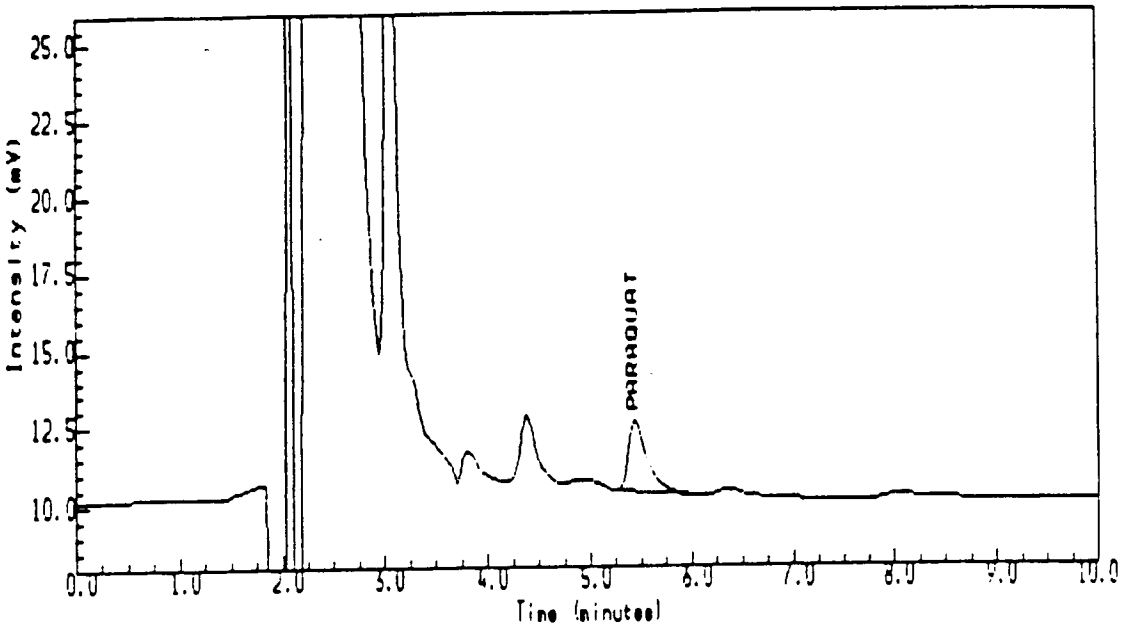
VOL I

FIgure 4

[RESIDUE] 23 IL277B,5,1
Reported on 14-NOV-1988 at 11:20

Injection Report

Acquired on 4-Jun-1988 at 13:27



Sample Name : 2640/88
Sample Id : 0
Sample Type : Sample Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	5.440	28964	0.039	PARAQUAT
Residual		0	N/A	
Total		28964	0.039	

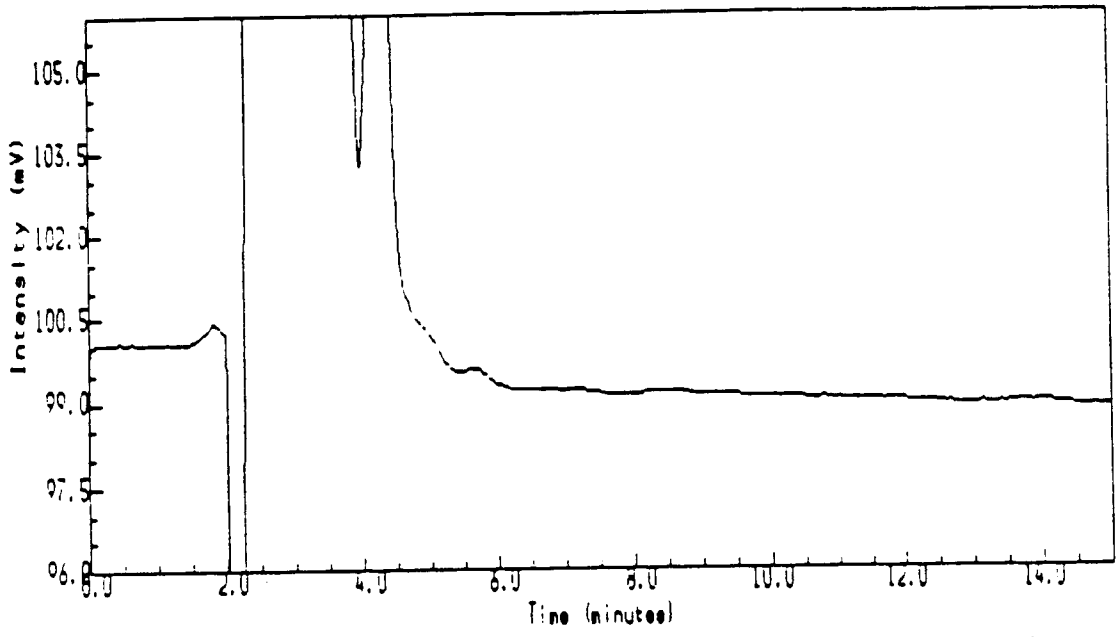
MISSING PEAKS

No missing peaks.

[RESIDUE] 23 DA402A,2,1
Reported on 14-NOV-1988 at 11:21

Injection Report

Acquired on 12-Oct-1988 at 01:24 by user T5



Sample Name : C3390/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 2

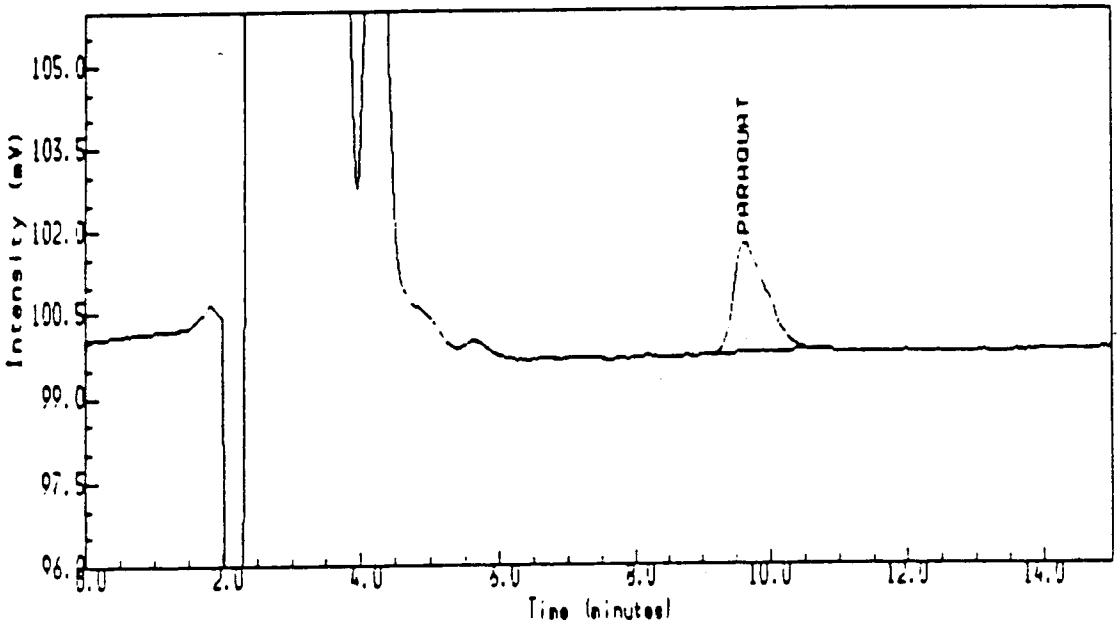
No peaks detected

Figure 6

[RESIDUE] 23 DA402A,3,1
Reported on 14-NOV-1988 at 11:23

Injection Report

Acquired on 12-Oct-1988 at 01:48 by user T5



Sample Name : R1 3390/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 3

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.643	65572	0.084	PARAQUAT
Residual		0	N/A	
Total		65572	0.084	

MISSING PEAKS

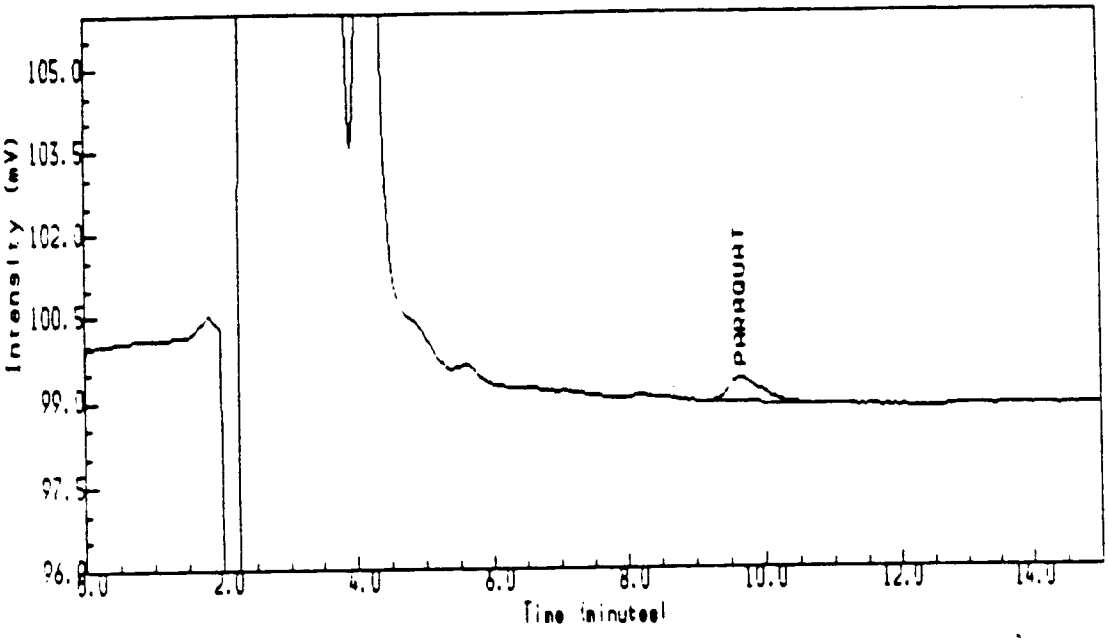
No missing peaks.

FIgure 7

[RESIDUE] 23 DA402A,4,1
Reported on 14-NOV-1988 at 11:23

Injection Report

Acquired on 12-Oct-1988 at 02:12 by user T5



Sample Name : 3385/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 4

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.685	14156	0.018	PARAQUAT
Residual		0	N/A	
Total		14156	0.018	

MISSING PEAKS

No missing peaks.

5.2 **Paraquat Residue Determination in Fat (Hen) and Heart (Sheep)**

Figure 1 : 0.1 μg cm^{-3} Paraquat

Figure 2 : Untreated fat sample at 0.5 g cm^{-3}

Figure 3 : Untreated fat smaple + 0.1 mg kg^{-1} at 0.5 g cm^{-3}

Figure 4 : Treated fat sample at 0.5 g cm^{-3}. Residue <0.005 mg kg^{-1}

Figure 5 : Untreated heart sample at 0.5 g cm^{-3}

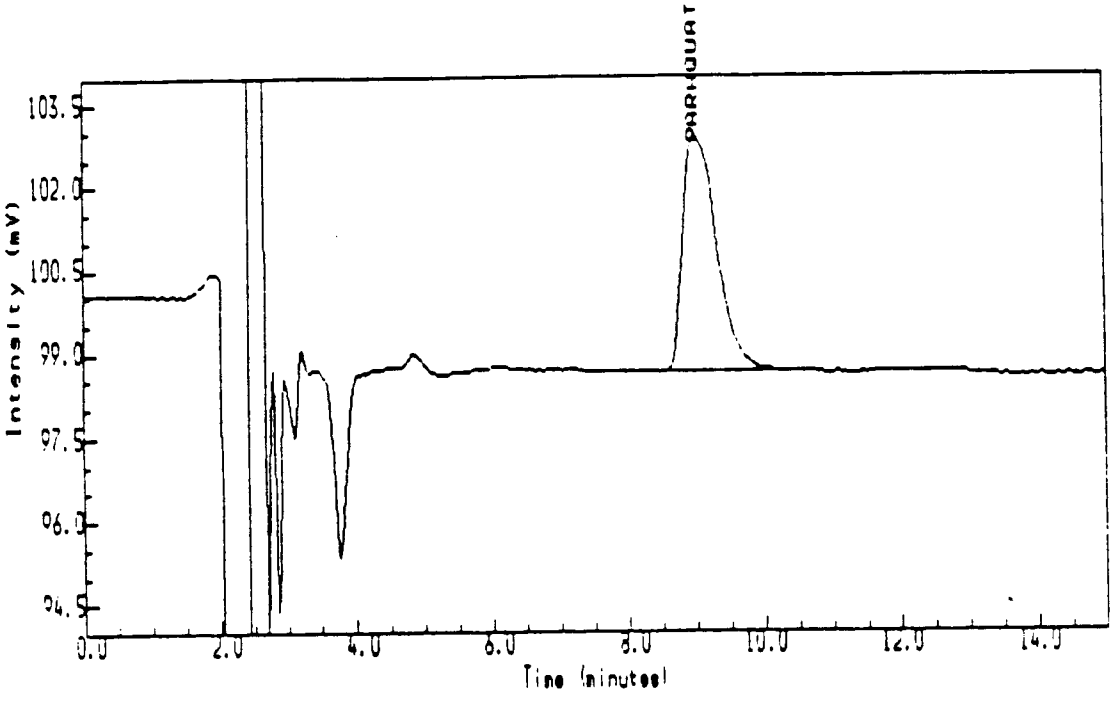
Figure 6 : Untreated heart sample + 0.1 mg kg^{-1} at 0.5 g cm^{-3}

Figure 1

[RESIDUE] 23 DA424A,1,1
Reported on 14-NOV-1988 at 11:25

Injection Report

Acquired on 20-Oct-1988 at 10:29 by user T5



Sample Name : STD
Sample Id :
Sample Type : Standard Amount=1.00000
Bottle No : 1

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.003	145957	0.098	PARAQUAT
Residual		0	N/A	
Total		145957	0.098	

MISSING PEAKS

No missing peaks.

Figure 2

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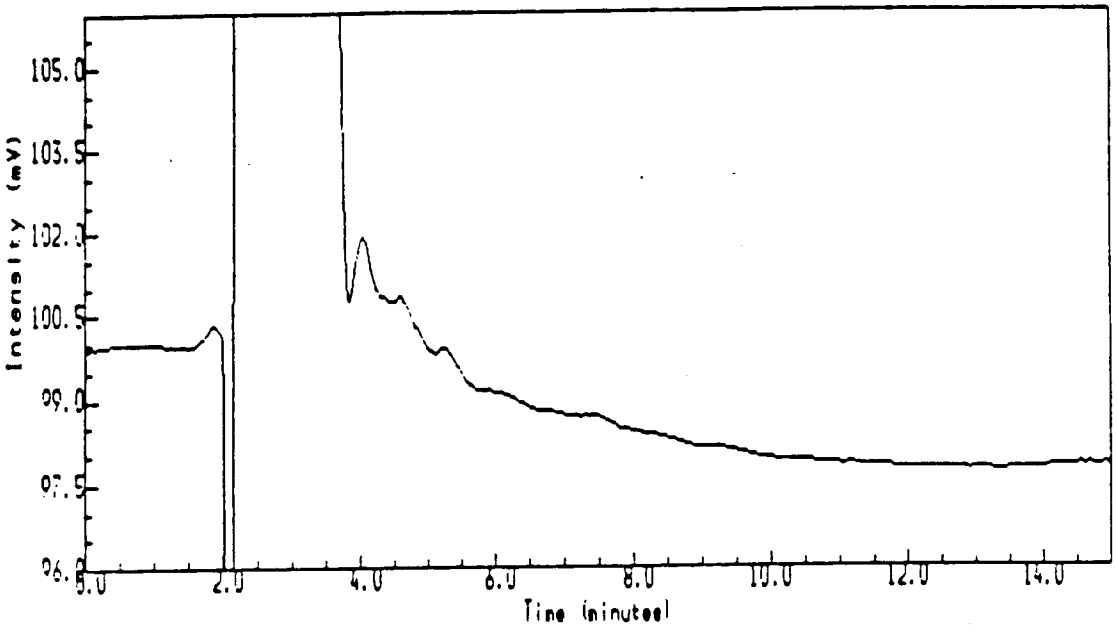
[RESIDUE] 23 DA425A,2,1
Reported on 14-NOV-1988 at 11:31

Injection Report

Acquired on 20-Oct-1988 at 16:25 by user T5



Sample Name : CONTROL
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 2

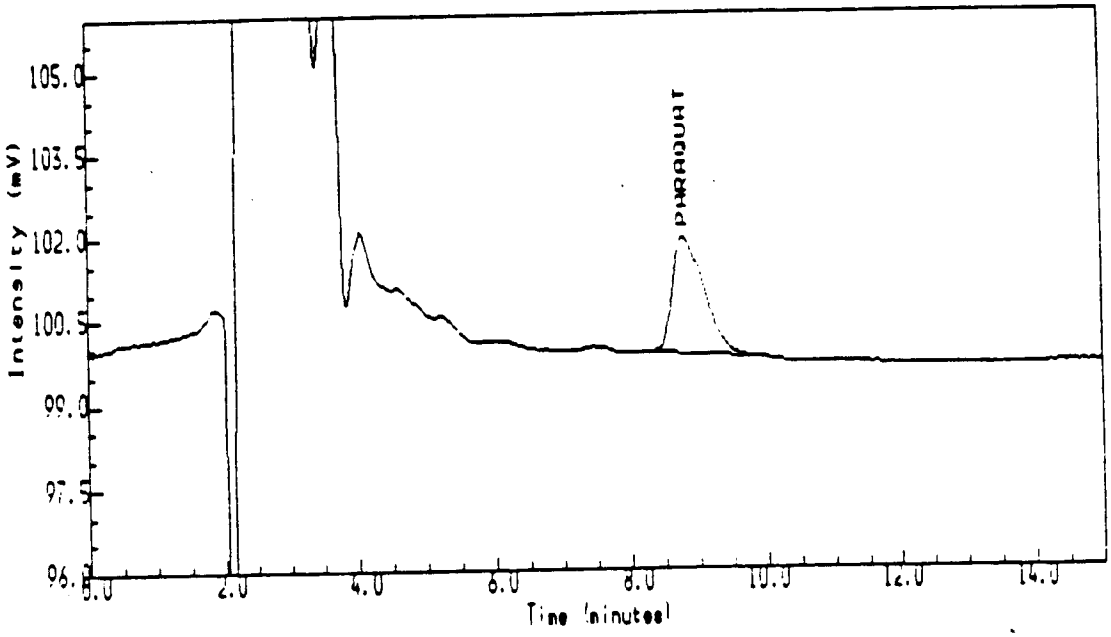
No peaks detected

Figure 3

[RESIDUE] 23 DA425A,3,1
Reported on 14-NOV-1988 at 11:29

Injection Report

Acquired on 20-Oct-1988 at 16:44 by user T5



Sample Name : R1 RECOVERY
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 3

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	8.811	67168	0.090	PARAQUAT
Residual		0	N/A	
Total		67168	0.090	

MISSING PEAKS

No missing peaks.

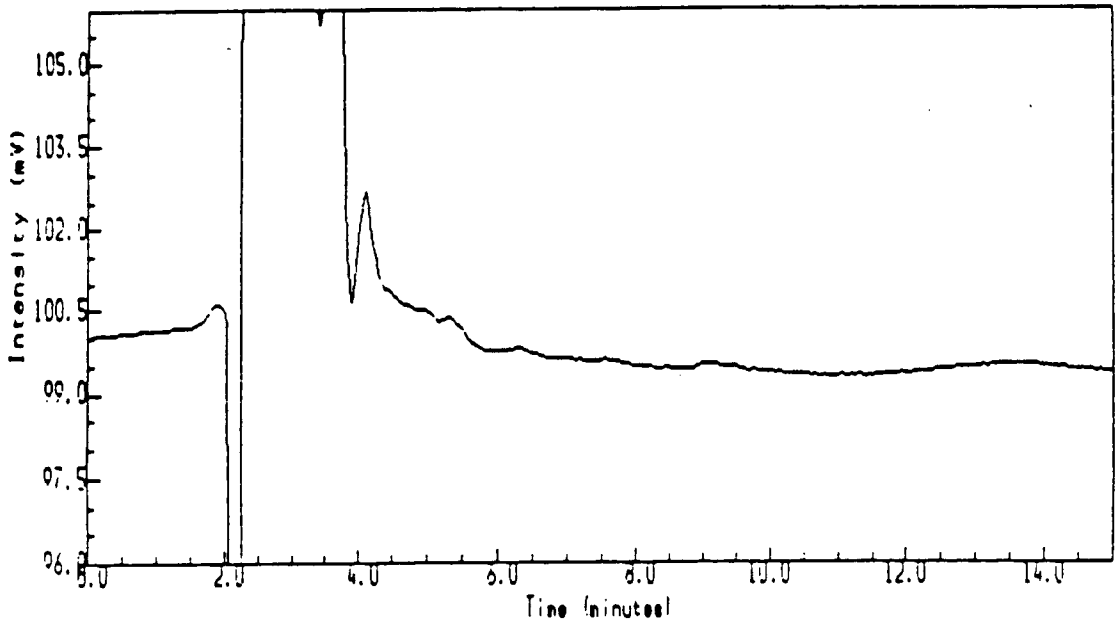
Figure 4

[VOL I 27

[RESIDUE] 23 DA425A,4,1
Reported on 14-NOV-1988 at 11:31

Injection Report

Acquired on 20-Oct-1988 at 17:03 by user T5



Sample Name : D2A
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 4

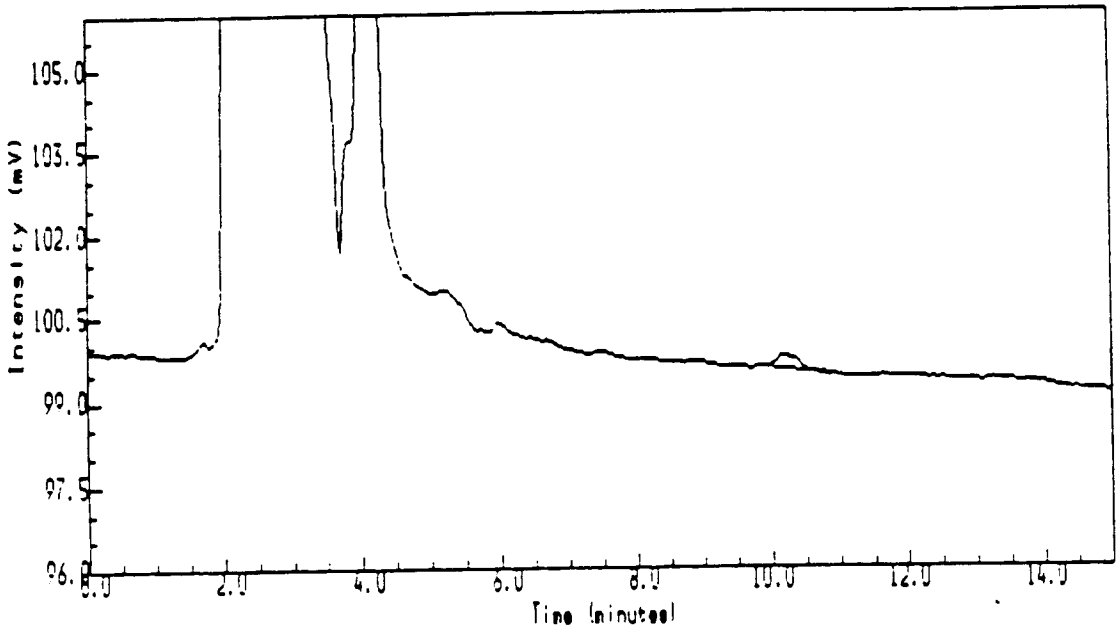
No peaks detected

Figure 5

[RESIDUE] 22 DA454C,2,1
Reported on 14-NOV-1988 at 11:34

Injection Report

Acquired on 7-Nov-1988 at 15:07 by user T6



Sample Name : C7080/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 2

PEAK INFORMATION

Peak RT mins	Area uVs	mg/kg	Peak name
Residual	5738	N/A	
Total	0	0.000	

MISSING PEAKS

RT mins	Peak name
9.600	[illegible]

Figure 6

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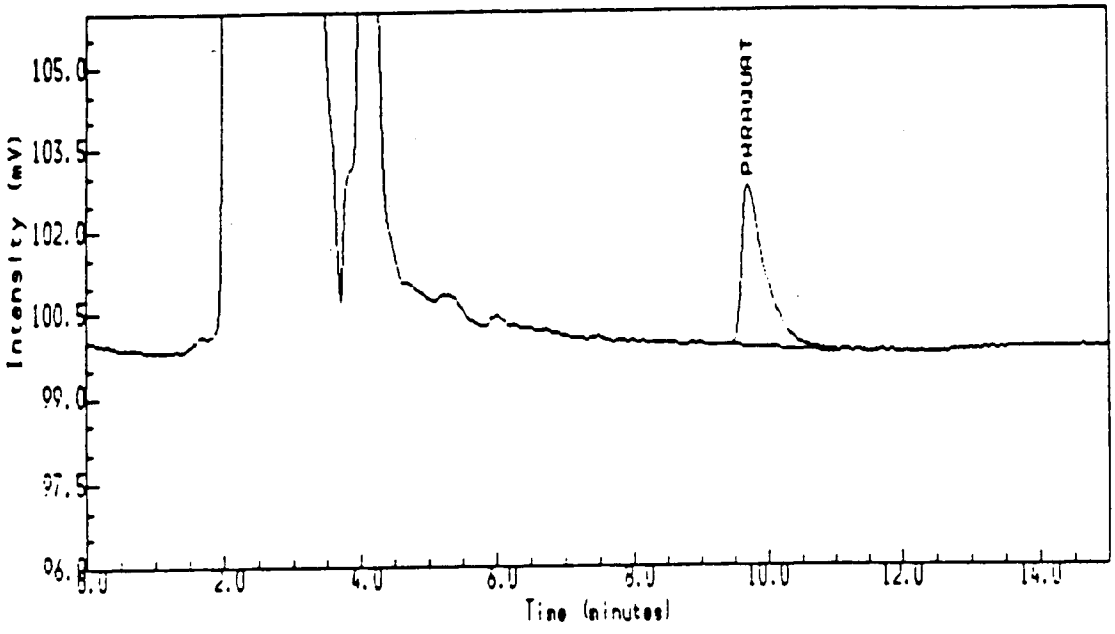
[RESIDUE] 22 DA454C,3,1
Reported on 14-NOV-1988 at 11:32

Injection Report

Acquired on 7-Nov-1988 at 15:32 by user T6



Sample Name : R1 7080/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 3

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.728	69533	0.090	PARAQUAT
Residual		0	N/A	
Total		69533	0.090	

MISSING PEAKS

No missing peaks.

5.3 Paraquat Residue Determination in Liver (Bovine) and Kidney (Pig)

Figure 1 : 0.1 $\mu g\ cm^{-3}$ Paraquat

Figure 2 : Untreated liver sample at 0.5 $g\ cm^{-3}$

Figure 3 : Untreated liver sample + 0.1 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 4 : Untreated kidney sample at 0.5 $g\ cm^{-3}$

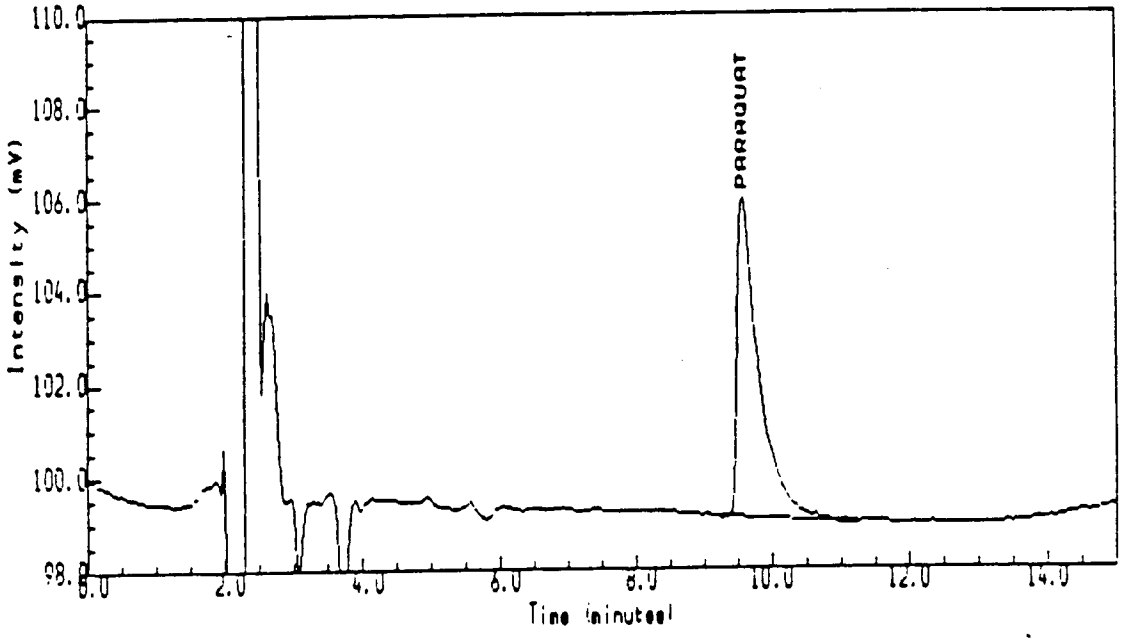
Figure 5 : Untreated kidney sample + 0.1 $mg\ kg^{-1}$ at 0.5 $g\ cm^{-3}$

Figure 1

[RESIDUE] 22 DA454C,9,1
Reported on 14-NOV-1988 at 11:40

Injection Report

Acquired on 7-Nov-1988 at 18:03 by user T6



Sample Name : STD
Sample Id :
Sample Type : Standard Amount=1.00000
Bottle No : 9

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.600	150383	0.097	PARAQUAT
Residual		0	N/A	
Total		150383	0.097	

MISSING PEAKS

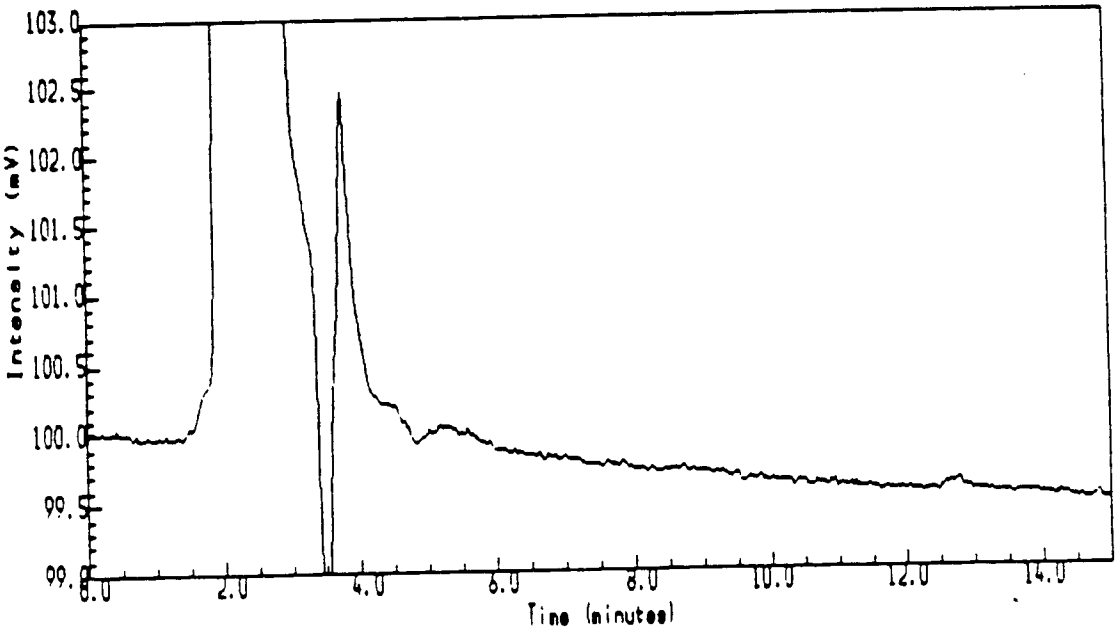
No missing peaks.

Figure 2

[RESIDUE] 22 DA459E,2,1
Reported on 21-NOV-1988 at 16:56

Injection Report

Acquired on 18-Nov-1988 at 17:33 by user T7



Sample Name : C7081/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 2

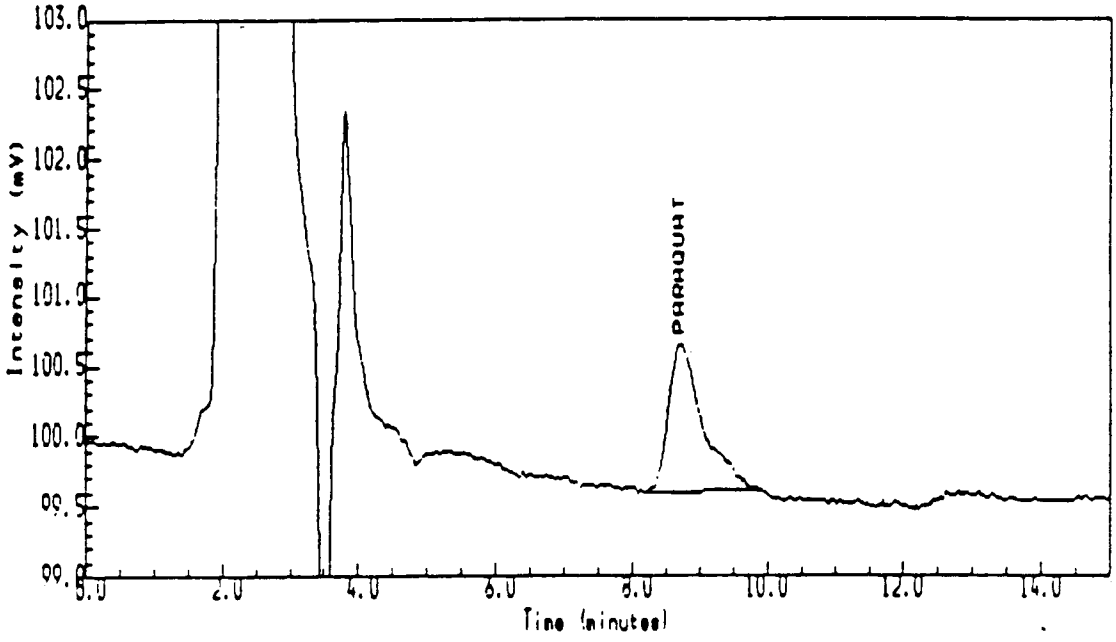
No peaks detected

Figure 3

[RESIDUE] 22 DA459E,3,1
Reported on 21-NOV-1988 at 16:55

Injection Report

Acquired on 18-Nov-1988 at 17:54 by user T7



Sample Name : R17081/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 3

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	8.704	37324	0.083	PARAQUAT
Residual		0	N/A	
Total		37324	0.083	

MISSING PEAKS

No missing peaks.

Figure 4

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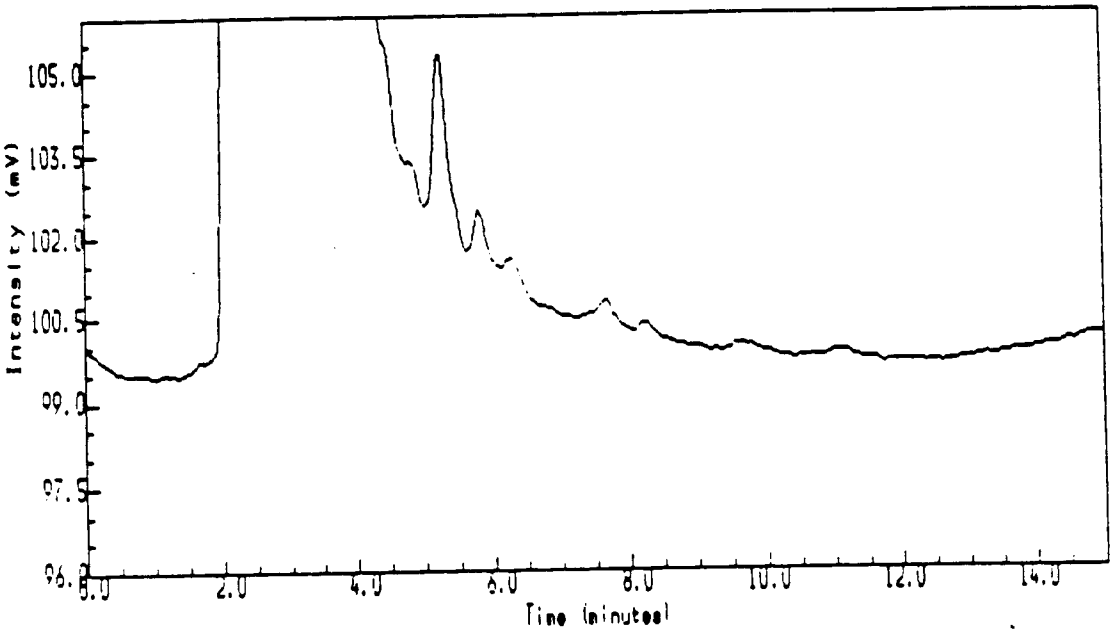
[RESIDUE] 22 DA454C,7,1
Reported on 14-NOV-1988 at 11:36

Injection Report

Acquired on 7-Nov-1988 at 17:13 by user T6



Sample Name : C7082/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 7

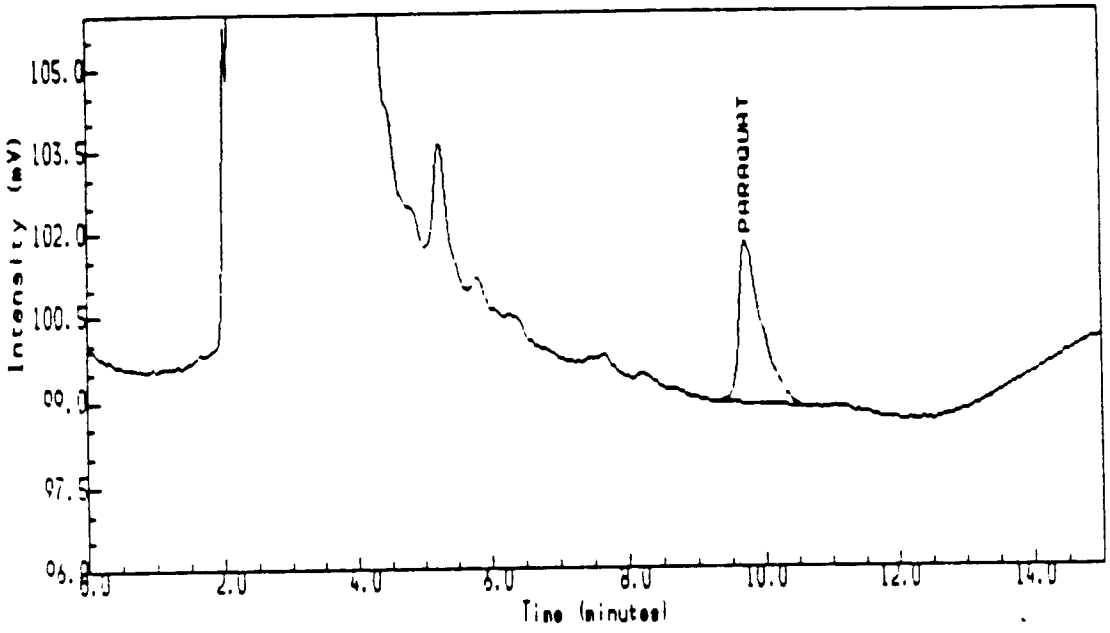
No peaks detected

Figure 5

[RESIDUE] 22 DA454C,8,1
Reported on 14-NOV-1988 at 11:37

Injection Report

Acquired on 7-Nov-1988 at 17:38 by user T6



Sample Name : R1 7082/88
Sample Id :
Sample Type : Sample Amount=1.00000
Bottle No : 8

PEAK INFORMATION

Peak	RT mins	Area uVs	mg/kg	Peak name
1	9.749	67074	0.087	PARAQUAT
Residual		0	N/A	
Total		67074	0.087	

MISSING PEAKS

No missing peaks.